Commentary

A *Tad* of dexterity: Did the Neurospora transposon use chromosome rearrangements to evade repeat-induced point mutation in Adiopodoume?

Adiopodoume (now Abidjan), in the Ivory Coast, West Africa, is a place in which an author might choose to locate her murder mystery merely on account of its name ("Prophets of Doume"?). However to Neurospora researchers Adiopodoume has become quite familiar, though not any less mysterious, because a *Neurospora crassa* strain isolated from there in 1955 was reported ten years ago to contain active copies of a LINE-like transposon, named *Tad* (Kinsey and Helber 1989). Few expected to find any repeated DNA, not to mention an active transposon, in the *N. crassa* genome because of the operation in the sexual stage of this fungus of a unique and aptly named mutagenic process called repeat-induced point mutation (RIP) (see Selker 1990 for a review). RIP efficiently detects linked or unlinked duplications of DNA segments and riddles both copies with multiple G : C to A : T transition mutations. Often, it also methylates many of the remaining cytosine residues. Thus unlike most eukaryotes, *N. crassa* has little redundant DNA other than its rRNA and tRNA genes (Krumlauf and Marzluf 1980). If sex is unsafe for repeated DNA in Neurospora, what made *Tad* tick in Adiopodoume?

Three possibilities were considered by Kinsey *et al* (1994): (i) That *Tad* had entered the *N. crassa* genome only very recently and it was doomed to imminent RIP. (ii) That *Tad* is inherently resistant to RIP. (iii) That the Adiopodoume strain is deficient in RIP. At first hypothesis (i) did look plausible. *Tad* was discovered by trapping two spontaneous mutants in the *am* (glutamate dehydrogenase) gene. These mutants were selected from the vegetative spores (conidia) of F_1 hybrids from a cross between Adiopodoume and a laboratory strain. The *am* gene had been previously cloned therefore the *Tad* sequences could be detected as novel 7 kb insertions in the mutant alleles, isolated, and used as a probe in Southern analysis. Multiple copies of *Tad* were detected in Adiopodoume but not in any of the > 400 other Neurospora strains examined, including other strains from West Africa and elsewhere (Kinsey 1989). These results thus supported hypothesis (i). However when the stringency of the Southern analysis was reduced, additional *Tad*-like sequences were detected not only in Adiopodoume but also in strains that were otherwise devoid of *Tad*. The *Tad*-like sequences were amplified by PCR with degenerate primers, cloned, and their partial nucleotide sequences were determined. These elements were clearly related to *Tad* but their sequences differed from that of *Tad* by 7% to 32% in the segments examined. The overwhelming majority of the sequence differences were of the type expected from the action of RIP on *Tad* (G : C to A : T transitions). *Tad*-related elements were also found in other Neurospora species, both heterothallic and homothallic. Thus all Neurospora species, or a common ancestor, appear to have hosted *Tad* and almost all copies of this transposon seemed to have been inactivated by RIP. These results argued against hypotheses (i), though it still remained formally possible that the active copies represent a "second coming" of *Tad* by a new horizontal transfer into Adiopodoume from some other organism.

The discovery of *Tad*-related elements elsewhere also threatened hypothesis (ii) although it was remotely conceivable that active *Tad* elements may be resistant to RIP. To test this, Kinsey *et al* (1994) took advantage of the unstable $Am^{+/-}$ phenotype of one of the mutants in which *Tad* had been trapped, *am* :: *Tad3-2* (referred to hereafter as 3-2). In this mutant the transposon was inserted into the 5′ noncoding sequence and the unstable phenotype was seemingly due to intermittent interference of *am*

transcription by *Tad*. This *Tad* element (*Tad3-2*) was active. In forced heterokaryons constructed between a naive strain and transformants bearing *Tad3-2*, the *Tad* sequences could transpose into nuclei of the naive strain (Cambareri *et al* 1994). Although most sexual progeny harbouring 3-2 continued to show the unstable $Am^{+/-}$ phenotype, some were stably Am^{+} and other were stably Am^{-}. Sequencing of a segment from a fragment spanning the *am-Tad3-2* junction from the Am^{+} progeny demonstrated that the loss of the unstable phenotype was indeed due to RIP-induced modification of *Tad3-2*. This showed that an active element is not spared by RIP.

Is Adiopodoume unable to RIP? To address this question Kinsey *et al* (1994) transformed the Adiopodoume strain with the plasmid pES201 which contained the bacterial *hph* (hygromycin phosphotransferease) gene driven by the *Aspergillus nidulans trpC* promoter and the *N. crassa am* gene driven by its own promoter. Transformants were selected on hygromycin-medium and one (referred to as T-2) was identified as having a single copy of the transforming DNA integrated at a site unlinked to the *am* gene. Note that this transformant contains two copies of the am^{+} gene (and is Am^{+}). When transformant T-2 was crossed to a series of other am^{+} strains, including the standard Oak Ridge strain ORSa, numerous Am^{-} progeny were produced amongst the progeny. This suggested that if Adiopodoume was defective in RIP this trait must be recessive. A second set of crosses was performed with the T-2 transformant (which had the *mat A* mating type of Adiopodoume) and 10 F_1 progeny of *mat a* mating type from a cross of ORSa × Adiopodoume, and the progeny were screened for *am* mutants. All 10 crosses produced am^{-} progeny which effectively ruled out the possibility that Adiopodoume contained a recessive mutation that caused a defect in RIP. The survival of *Tad* in Adiopodoume remained unexplained.

As in all good mysteries a possible clue to *Tad*'s survival may have lain hidden at the beginning of the story after all. The first paper on *Tad* reported a personal communication from David Perkins wherein he observed ". . . that translocations were probably more frequent in crosses involving Adiopodoume than in crosses involving only standard lab strains . . .". Kinsey and Helber (1989) speculated that this higher frequency of spontaneous chromosomal aberrations might reflect the ectopic pairing and recombination between *Tad* elements at different chromosomal sites in Adiopodoume. But might the rearranged chromosomes also have a role in protecting *Tad* from RIP?

In crosses heterozygous for translocation chromosomes, the segregation of translocation chromosomes with normal chromosomes in meiosis can produce progeny that are now duplicated for the translocated segment. Depending on the size of the translocations, the segmental duplications can be quite large (e.g., > 100 kb). Perkins *et al* (1997) showed that RIP could also occur in these large chromosome segment duplications but its efficiency appeared to be reduced and when it did occur, the mutagenesis seemed milder than that typically induced by gene-sized duplications. Ashwin Bhat in our laboratory decided to examine whether a large chromosome segment duplication also affected the ability of a small duplication in the same nucleus to induce RIP in its target gene. He found a dramatic decrease in the induction of RIP in *erg-3* by an ectopically integrated 1.3 kb duplicated fragment in nuclei that also contained the large chromosome segment duplication *Dp(IIIR > [IR; IIR])AR17* (A Bhat and D P Kasbekar, unpublished results). This suggested that small duplications can escape RIP if a large duplication is present in the same nucleus. Thus *Tad* would have enjoyed RIP-free passage in ancestral strains of Adiopodoume that contained one or more large chromosome segment duplications. Some of the translocation chromosomes detected by Perkins in Adiopodoume might indeed represent elements of such ancestral duplications.

However the answer might not be quite so simple. Crosses involving segmental duplication strains are characteristically barren, i.e., only a few exceptional asci produce a few viable ascospores. Whereas euploid strains (normal sequence and translocation) exhibit high RIP efficiency and are productive, duplication strains (that are obtainable from crosses between normal sequence and translocation strains) exhibit low RIP but are relatively unproductive. *Tad*'s survival in Adiopodoume might reflect an adroitness in switching between duplication and euploid host nuclei. One way of doing so would be by transposition in fortuitously formed heterokaryons. Another would be to take advantage of duplication instability. Many Neurospora duplications breakdown during vegetative growth by loss of the duplicated segment either from the translocated or the normal position and thereby restore euploidy (see Perkins 1997 for a review). Loss from the translocated position restores the normal sequence and

occurs more frequently than loss from the normal position, which restores the translocation sequence. Turner (1977) reported an intriguing exception where the loss from either position is equally likely.

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